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Variability in growth/no growth boundaries of 188 different *Escherichia coli* strains reveals that approximately 75 % have a higher growth probability under low pH conditions than *E. coli* O157:H7 strain ATCC 43888



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ABSTRACT

This study investigated the variation in growth/no growth boundaries of 188 *Escherichia coli* strains. Experiments were conducted in Luria–Bertani media under 36 combinations of lactic acid (LA) (0 and 25 mM), pH (3.8, 3.9, 4.0, 4.1, 4.2 and 4.3 for 0 mM LA and 4.3, 4.4, 4.5, 4.6, 4.7 and 4.8 for 25 mM LA) and temperature (20, 25 and 30 °C). After 3 days of incubation, growth was monitored through optical density measurements. For each strain, a so-called *purposeful selection approach* was used to fit a logistic regression model that adequately predicted the likelihood for growth. Further, to assess the growth/no growth variability for all the strains at once, a generalized linear mixed model was fitted to the data. Strain was fitted as a fixed factor and replicate as a random blocking factor. *E. coli* O157:H7 strain ATCC 43888 was used as reference strain allowing a comparison with the other strains. Out of the 188 strains tested, 140 strains (~75%) presented a significantly higher probability of growth under low pH conditions than the O157:H7 strain ATCC 43888, whereas 20 strains (~11%) showed a significantly lower probability of growth under high pH conditions.

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1. Introduction

Escherichia coli is naturally present in the gastrointestinal tract of humans and other animals and, in general, is not harmful to the host. Nonetheless, certain *E. coli* strains have acquired specific virulence genes which induce the ability to cause a large number of diseases (Ahmed et al., 2008). Two factors allow *E. coli* to acquire and lose those virulence genes at a relatively high frequency: the high plasticity of the genome and the fact that most of the virulence genes are encoded in mobile elements such as plasmids, phages or transposons (Kuhnert et al., 2000). Gene transfer occurs in many environments leading to strains with new combinations of virulence genes that might emerge in the future (Kaper et al., 2004; Kelly et al., 2009).

E. coli O157:H7 is the major food-borne pathogen linked to outbreaks related to ground beef products (Doyle, 1991). The main reservoir of this bacteria is known to be the bovine gastrointestinal tract (Price et al., 2004). However, many outbreaks were also linked to other foods types including acid foods like mayonnaise (Weagant et al., 1994), apple cider (Zhao et al., 1993) and yogurt (Morgan et al., 1993). The mechanisms used by *E. coli* to survive in acidic environments involve an increase of internal pH and a change in both transmembrane electrical potential and metabolic activity (Foster, 2004).

Knowledge of microorganism growth limits under different environmental conditions allows for a better quality and safety management of foods (McMeekin et al., 2000). Models used to define the growth limits are known as growth/no growth interface, growth boundary or growth limit models (Ross and Dalgaard, 2004). Growth/no growth models can predict suitable combinations of hurdles making microbial growth highly unlikely (Masana and Baranyi, 2000). Different methodologies to observe *E. coli* growth boundaries under different combinations of environmental

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factors can be found in literature (McKellar and Lu, 2001; Presser et al., 1998; Salter et al., 2000; Skandamis et al., 2007; Valero et al., 2010). Most studies consider either a limited number of strains or a mix of strains. Valero et al. (2010) performed experiments using four different *E. coli* serotypes at different temperature, pH and inoculum levels. McKellar and Lu (2001) observed the growth boundaries for a mix of five *E. coli* O157:H7 strains according to temperature, pH and concentration of acetic acid, salt and sucrose, in a system mimicking a mayonnaise sauce. Skandamis et al. (2007) modeled the growth boundaries of nonadapted and acid-adapted *E. coli* O157:H7 (mixture of 4 strains) influenced by pH, NaCl concentration and temperature.

Since variability between strains may have an important impact on the accuracy of risk assessment outcomes (Lianou and Koutsoumanis, 2013), information about the variability in phenotypic responses among strains of the same species under different environmental conditions is crucial (Nauta and Dufrenne, 1999). The variability between strains of the same species is discussed in the literature through several biological observations, like variability in heat resistance, biofilm formation, growth behavior and acid resistance. So far, however, a limited amount of data regarding the variability in growth/no growth boundaries among different strains is available in literature (Lianou and Koutsoumanis, 2013). Experiments on the growth kinetics of 17 *E. coli* O157:H7 strains in brain heart infusion adjusted to pH 5.3 with lactic acid demonstrated that the lag phase could vary from 13.7 to 55.6 h (Whiting and Golden, 2002). Heat inactivation at 60 °C in a simulated beef gravy medium of five *E. coli* serotypes (O157:H7 and non-O157:H7) from clinical and food isolates showed a significant difference in survival curves (Juneja and Marks, 2005).

Mixed-effects modeling is a statistical approach to model hierarchical data structures by clustering observations into groups that may arise from repeated measurements on the same strains or individuals (Schielzeth and Nakagawa, 2013). Despite being a state of the art statistical approach, mixed-effects models are not frequently used in predictive microbiology, exceptions being Juneja and Marks (2005), Krulikosj et al. (2011), Mand et al. (2013) and Shorten et al. (2004). In mixed-effects models two types of effects are considered to model the data: the *fixed effects*, whose levels are experimentally determined such as temperature, pH, selected strain, and the *random effects*, whose levels are sampled from a large population and are due to biological variability (Bolker et al., 2009). Another definition for fixed and random effects is that fixed effects are related to unknown parameters to be estimated from data and random effects govern the variance-covariance structure of the independent variable (Crawley, 2007). For example, random effects include variation among individuals when multiple responses are measured per individual, region, genotype or species. Fixed effects are indeed the variables included in the statistical model, but random effects are strictly speaking not variables but unobserved random variation (Bates, 2010). Generalized linear mixed model (GLMM) combines the properties of two statistical approaches: mixed-effects models and generalized linear models. Generalized linear models (GLM) are a generalization of the ordinary linear regression but allowing a linear model to be related to response variables that are not normally distributed through the use of an appropriate link function, e.g. the logit link $\log(p/1-p)$ in the case of logistic regression, where a binomially distributed dependent variable, with probability of occurrence p , is related to one or more continuous covariates.

The objectives of this study were (a) to observe the growth/no growth interfaces of 188 *E. coli* strains isolated from different sources, (b) to model the growth/no growth interfaces of each *E. coli* strain with a logistic regression technique, (c) to model the variation among the strains and assess the level of variability among

them through the use of GLMM, and (d) to assess whether highly resistant *E. coli* strains can be isolated from the environment.

2. Material and methods

2.1. Bacterial strains and inoculum preparation

The collection of *E. coli* strains comprised 18 avian pathogenic *E. coli* (APEC) strains obtained from Prof. B. Goddeeris (KU Leuven, Belgium), 20 cytotoxic necrotizing factor (CNF)-producing (type 1 and 2), 8 necrotoxic (NETEC II), 14 enteropathogenic (EPEC) and 8 enterotoxigenic (ETEC) strains from Prof. J. Mainil (Ulg, Liège, Belgium), 20 strains from the *E. coli* Reference Collection (ECOR) (<http://www.shigatox.net/new/reference-strains/ecor.html>), 97 strains from the Laboratory of Food Microbiology (KU Leuven, Belgium) collection isolated from diverse environment sources (coded as EC or BV followed by a number), *E. coli* O55:H5 (ATCC 12014), O29 (ATCC 43892), O157:H7 (ATCC 43888) and MG1655. Stock cultures were maintained at –80 °C in Luria–Bertani media (LB) with 25% vol/vol glycerol. Active cultures of each strain were obtained by streaking a loopful of the frozen stock cultures into stock plates (LB agar plates) followed by incubation for 24 h at 37 °C. Stock plates were kept at 4 °C and redone every 2 weeks. Pre-inoculum cultures were obtained by picking one single colony from the stock plates and incubating it using LB media (pH 7) into microtiter plates overnight at 37 °C.

2.2. Experimental design

Growth/no growth interfaces were evaluated with respect to temperature (20, 25 and 30 °C), pH (from 3.8 to 4.8) and presence of LA (0 and 25 mM). The LB media pH with 25 mM of LA was adjusted with NaOH 1 M in six levels: from 4.3 to 4.8 with increments of 0.1 pH units. The LB media pH without LA (0 mM) was adjusted with HCl 37% also in six levels: from 3.8 to 4.3 with increments of 0.1 pH units. For all conditions pH was aseptically adjusted using a digital pH meter (Hanna, HI9125) after autoclaving. The HCl and NaOH solutions were filter sterilized before use. In total, 36 conditions per strains and four biological replicates (four different single cell colonies from the activated cultures in LB agar plates) per condition were tested. For half of the strains, the four biological replicates were extended to eight biological replicates at 20 °C, 0 mM LA and pH from 3.8 to 4.0. For *E. coli* O157:H7 strain ATCC 43888, eight biological replicates for all conditions and 12 biological replicates at 20 °C, 0 mM LA and pH from 3.8 to 4.0 were tested.

2.3. Growth/no growth experiments

Pre-inoculum cultures were diluted in LB media with adjusted pH, added to microtiter plates, to reach an initial inoculum of approximately 10^5 CFU/ml. All microtiter plates were sealed with a special cover (Enzymscreen, <http://www.enzymscreen.com>) that limits the evaporation and ensures equal oxygen conditions in all wells. Afterward the sealed microtiter plates were placed into clamp systems (Enzymscreen, <http://www.enzymscreen.com>) mounted on orbital shakers and incubated at the respective temperature. After three days of incubation the optical density (OD) was measured at 600 nm (Multiskan RC, Thermo Labsystems). Growth was considered to have occurred when the OD was higher than 0.150. This value was chosen during preliminary experiments by generating a growth curve at pH 7 and 37 °C for *E. coli* MG1655 correlating OD measurements and number of cells by plate counting. No subsequent plating to check for contamination was done. Instead, one of the microtiter plates' wells was not inoculated (blank) and if it did

not show growth, no contamination was considered for the entire microtiter plate.

2.4. Logistic regression model development

Logistic regression describes the relationship between the probability p of event success, in this case bacterial growth, and a set of independent variables, in this case pH, temperature, and lactic acid concentration. The logistic regression used to describe the growth response, per strain, according to the temperature (T), pH and total lactic acid (LA) was:

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 \cdot pH + \beta_2 \cdot T + \beta_3 \cdot LA + \beta_4 \cdot pH \cdot LA + \beta_5 \cdot pH \cdot T + \beta_6 \cdot T \cdot LA \quad (1)$$

where p is the probability of growth and β_i are the coefficients to be estimated.

The traditional approach used so far in predictive microbiology to select the logistic regression variables to model the growth/no growth boundaries involves automated model selection procedures available in commercial software such as SAS (as in Vermeulen et al., 2009), STATISTICA (as in Arroyo López et al., 2007) and SPSS (as in Valero et al., 2010). During preliminary tests, however, it was observed that the use of automated model selection procedures (e.g. based on minimizing the Akaike Information Criterion (AIC) or selection of variable with p -values lower than 0.05 or 0.01) did not lead to biologically plausible models for some of the strains. Therefore, the method for selecting the model variables for each strain was an adaptation of the method of purposeful selection, suggested by Hosmer et al. (2013), which is described in the following steps:

Step 1: First each variable was separately included in the model. Through the use of this univariable analysis variables were selected for a first multivariable model. The selection criteria to include the variables in the model at all steps was a p -value of the Wald statistic test lower than 0.05. In the original procedure, the p -value used for selection in the univariable analysis was 0.25 in order to not discard variables that might still have a biologically important influence on the outcome; we used the lower value of 0.05 because the number of variables in our experiments was lower than in the examples used by Hosmer et al. (2013).

Step 2: A multivariable model containing all variables identified in Step 1 was fitted.

Step 3: Each variable not selected in Step 1 was added to the model obtained in Step 2. This step was essential to identify variables that were not significantly correlated to the outcome by themselves but still made an important contribution to the model in the presence of other variables.

Step 4: Interactions among the variables selected in the previous steps were checked. The interactions were added one at a time to the main effects models from Step 3. Following, the interactions with a p -value lower than 0.05 were concomitantly included to the model.

Step 5: In some cases the inclusion of more than one interaction resulted in a model with no significant variables. In this case we returned to Step 4 and chose the best model containing the interaction variables with the lowest AIC-value. This step was not included in the original purposeful selection procedure.

The original purposeful selection includes more steps not needed in our study because of the number of measured variables.

The examples used by Hosmer et al. (2013) are related to clinical studies and include many more variables, leading to a more extensive selection procedure.

The parameters estimation via the purposeful selection method was carried out using the glm function in R (version 2.15.2, R Core Team, 2012). The significance of the obtained models was tested using the log-likelihood and the AIC value. Graphics containing the growth/no growth responses were generated in Matlab® Version 7.7 (The Mathworks, Inc., Natick, USA).

2.5. Comparison of models with independent data

The predictions of 10, 50 and 90% of growth probability calculated in this work for *E. coli* O157:H7 strain ATCC 43888 were compared with literature data using other representatives of O157:H7. The data sets were chosen because of their similarities regarding pre-inoculum, temperature and pH conditions. The growth/no growth responses used for comparison were: (1) a mix of five *E. coli* O157:H7 (C7927, C9490, 380–94, EC940340, and EC920283) in tryptic soy broth (TSB) for 72 h, with initial concentration of 10^7 CFU/ml, 0.5% of salt and 0% of acetic acid and sucrose (McKellar and Lu, 2001) and (2) a mix of four *E. coli* O157:H7 strains (ATCC 43895, ATCC 43889, ATCC 51658 and EO139) in TSB for 60 days, with initial inoculum of 3.4×10^5 CFU/ml, aw of 0.995, pH adjusted with LA, without previous acid adaptation (Skandamis et al., 2007).

2.6. Generalized linear mixed model

GLMMs were used to assess the variability in the growth/no growth responses between all 188 strains. From the tested strains O157:H7 strain ATCC 43888 was the most appropriate to be chosen as the reference strain since this was the only variant of this important food born pathogen studied. *E. coli* O157:H7 strain ATCC 43888 was isolated from human feces and does not contain the genes for Shiga-like toxin I or II production. The total number of observations for the entire experiment was enormous (approximately 4 biological replicates \times 6 pH \times 2 LA concentration \times 3 temperatures \times 188 strains = 27 072 observations). Hence, we decided to fit separate GLMMs to the data coming from 12 different treatment conditions (Table 1) each with fixed temperature and LA concentration, but including a range of pH values. In total, 12 GLMMs were fitted to the data. The different strains were considered as the fixed effect and the replicates nested within strains (1|Strain/Replicate), were considered as the random factor. Our choice of biological replicates as random effect implies that we consider the replicates as the factor that contributes the most to the observed variability in the growth response for a fixed condition. The GLMM was fitted to the data using R (version 2.15.2, R Core Team, 2012) with the glmer function from package lme4 (Bates, 2010), using a binomial logit response. The estimated coefficients, obtained through the GLMM on R for each strain, were

Table 1

Lactic acid concentration (LA), temperature (T) and pH range for the twelve conditions used to model the growth/no growth data using generalized linear mixed models (GLMM).

Cond.#	LA (mM)	T (°C)	pH range	Cond.#	LA (mM)	T (°C)	pH range
1	0	20	3.8–4.0	7	25	20	4.3–4.5
2	0	20	4.1–4.3	8	25	20	4.6–4.8
3	0	25	3.8–4.0	9	25	25	4.3–4.5
4	0	25	4.1–4.3	10	25	25	4.6–4.8
5	0	30	3.8–4.0	11	25	30	4.3–4.5
6	0	30	4.1–4.3	12	25	30	4.6–4.8

automatically calculated (by the glmer function) relatively to the reference strain through the use of the function `relevel`. Then, the odds ratio was calculated by the exponential of the estimated coefficient. For example, the estimated coefficient for EC1 at condition 1 was 3.17, the odds ratio was equal to 24.01 ($\exp(3.17)$). Graphics comparing the odds ratio were generated in R with the package `ggplot2`.

3. Results

3.1. Growth/no growth model for the 188 *E. coli* strain

The growth/no growth boundaries varied largely among the tested *E. coli* strains. Fig. 1 depicts the growth response and the predicted growth probability lines of 10, 50 and 90% for EC1, O157:H7, MG1655, EC26 and EC13. When growth occurred in some of the replicates a triangle is used followed by the % of replicates that grew, for most conditions this percentage of growth is 25, 50 or 75% as four replicates were tested, but for some strains like O157:H7 eight or twelve replicates were tested. The transition from

growth to no growth occurred gradually for the majority of the strains, e.g., EC1, O157:H7, MG1655 and EC13 showed in Fig. 1, and ECOR16, EC160, APEC150 and BV9 showed in the Supplementary file (S1). However, some strains, e.g., EC26 (Fig. 1), EC47, APEC10, APEC36 and BV25 (S1), showed an abrupt stop of growth between successive pH increments of 0.1 pH units in media containing 25 mM of LA.

Among the most resistant strains for the growth boundaries without LA are EC1, capable to grow at pH 3.8 at all tested temperatures, and EC13, capable to grow at pH 3.9 at all tested temperatures, while *E. coli* O157:H7 grew on 50% of the replicates at pH 3.9 and 25 °C. *E. coli* MG1655 and EC26 are examples of strains sensitive to low pH, both strains only presented growth at all three temperatures tested at pH 4.3. The behavior of the strains in Fig. 1 with 25 mM of LA is similar to the ones without LA. EC1 grew at 25 and 30 °C and pH 4.4, and EC13 grew at pH 4.3 and 4.4 at 25 °C, while MG1655 and EC26 grew at 30 °C and pH 4.6 and 4.7, respectively. In some strains a decrease in the growth probability is observed while the environmental factors became more favorable. EC26 at 30 °C has a growth probability of 25% at pH 3.9 and 0 mM

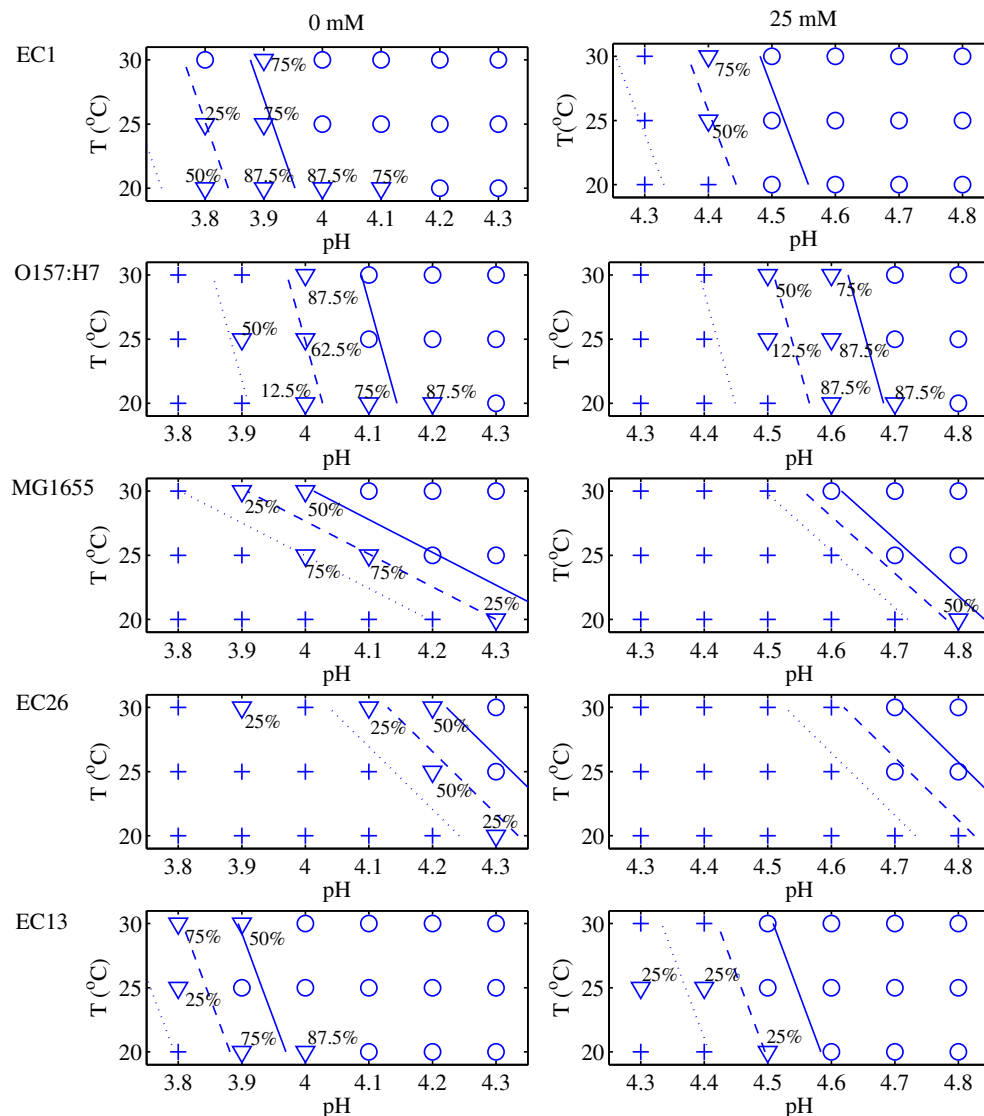


Fig. 1. Growth responses and predicted growth/no growth interfaces of *E. coli* EC1 (isolated from minced meat), O157:H7 strain ATCC 43888, MG1655, EC26 (isolated from soil) and EC13 (isolated from chicken feces) with 0 mM (left graphics) and 25 mM (right graphics) of lactic acid. Data points: (○) 100% of growth, (+) no growth and (▽) growth probability between 0 and 100%. Lines: predicted growth probability of 10% (····), 50% (----) and 90% (—).

		Intercept	pH	LA	T	pH.LA	pH.T	T.LA	AIC	Log-L
EC1	Parameter	-77.12	19.32	-0.47	0.15	n.s.	n.s.	n.s.	85.03	-38.51
	Std. Error	15.31	3.84	0.09	0.07					
	z value	-5.04	5.03	-5.12	1.98					
	p value	0.000	0.000	0.000	0.048					
O157:H7	Parameter	-77.79	18.79	-0.40	0.11	n.s.	n.s.	n.s.	181.08	-86.54
	Std. Error	8.83	2.13	0.05	0.05					
	z value	-8.81	8.83	-8.31	2.19					
	p value	0.000	0.000	0.000	0.029					
MG1655	Parameter	-106.29	20.91	-3.36	0.82	0.62	n.s.	n.s.	64.38	-27.19
	Std. Error	23.46	4.81	1.22	0.18	0.26				
	z value	-4.53	4.35	-2.75	4.59	2.38				
	p value	0.000	0.000	0.006	0.000	0.017				
EC26	Parameter	-112.54	23.71	-0.47	0.49	n.s.	n.s.	n.s.	64.15	-28.08
	Std. Error	23.75	5.12	0.10	0.12					
	z value	-4.74	4.63	-4.45	4.08					
	p value	0.000	0.000	0.000	0.000					
EC13	Parameter	-100.90	25.03	-0.61	0.19	n.s.	n.s.	n.s.	76.60	-34.30
	Std. Error	19.06	4.72	0.12	0.08					
	z value	-5.29	5.30	-5.27	2.31					
	p value	0.000	0.000	0.000	0.021					

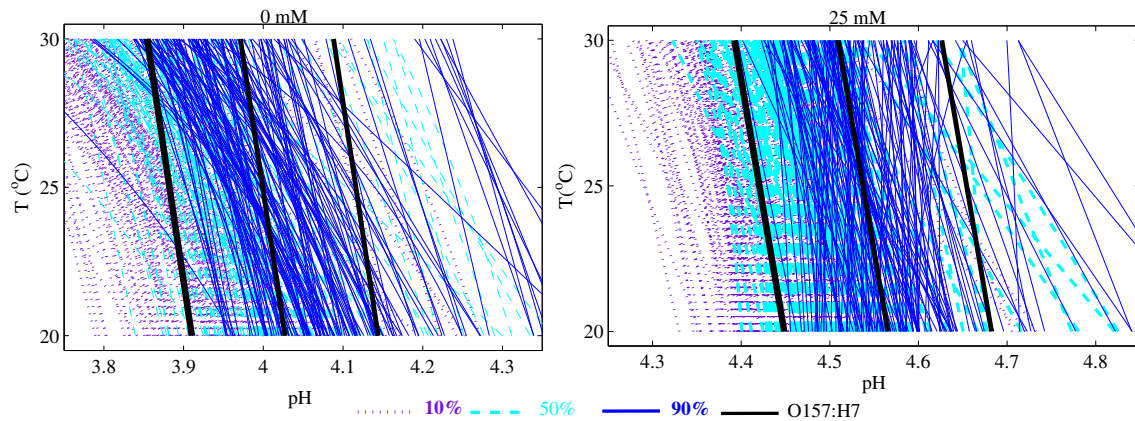


Fig. 2. Predicted growth/no growth interfaces with 0 and 25 mM of lactic acid for all *E. coli* strains which pH and temperature variables were significant in the logistic regression model fitted to the data. The thicker solid black lines represent the growth boundaries for *E. coli* O157:H7 strain ATCC 43888 of 10, 50 and 90% from left to right respectively.

the respective condition, and strains with odds ratio lower than one have a lower odds of growth than the reference.

For all conditions except for condition 7 (25 mM, 20 °C and pH 4.3–4.5) and 12 (25 mM, 30 °C and pH 4.6–4.8), the growth responses of *E. coli* strains were significantly different ($p < 0.05$) from O157:H7 strain ATCC 43888. The number of strains significantly different from O157:H7 strain ATCC 43888 with higher odds of growth (Fig. 4) was greater than the ones with lower odds of growth (Fig. 5). In total, 140 strains (~75%) showed higher odds of growth than *E. coli* O157:H7 strain ATCC 43888 in low pH conditions (3.8–4.0 without LA and 4.3–4.5 with LA). The odds of growth for *E. coli* EC1 was approximately 24 times higher than the odds of growth for *E. coli* O157:H7 in condition 1, and approximately 41 times higher in condition 5 (Fig. 4). For high pH ranges (4.1–4.3 without LA and 4.6–4.8 with LA), the odds of growth for 20 strains (Fig. 5) were lower than the reference. The odds of growth for *E. coli* BV23 was approximately 0.018 times lower than the odds of growth for *E. coli* O157:H7 in condition 2, and approximately 0.22 times lower in condition 8 (Fig. 5).

Some strains were significantly different from O157:H7 strain ATCC4388 in many conditions. BV23 (from horse feces), EC28 (from soil) and ECOR-08 (O86, from feces of a healthy person) grew less than the reference strain at five conditions (2, 4, 6, 8 and 10), all corresponding to high pH ranges (Fig. 5). Nine strains grew better than the reference strains at four conditions (1, 3, 5 and 9), all corresponding to low pH ranges, among them are EC1, EC13, EC3

(from minced meat), and ECOR-01 (from feces of a healthy person) (Fig. 4).

4. Discussion

This study analyzed the variability in the growth/no growth interfaces of 188 *E. coli* strains in LB media under 36 environmental conditions according to pH, temperature and presence of lactic acid. As in the majority of studies regarding strains variability in growth, inactivation, acid resistance among other treatments, our findings highlighted that heterogeneity on growth/no growth boundaries of *E. coli* strains from different sources is significant. This variability in growth, as defined by an OD increase of 0.15, maybe a function of differences between strains/repetitions in chances of initiating growth, in overpassing the lag phase and/or in the growth rate. As a consequence, this variability could probably be reduced if the time of incubation was larger than 3 days, giving the cells more chances to initiate growth.

The purposeful selection approach was an adequate alternative to describe the correlation between the variables pH, temperature and lactic acid with the growth/no growth response of *E. coli*. Interpretation of a logistic regression model or any fitted model requires the ability to make practical inferences from the estimated parameters for each variable in the model (Hosmer et al., 2013). As the significant variables selected through the purposeful selection varied among the *E. coli* strains, the interpretation for each strain

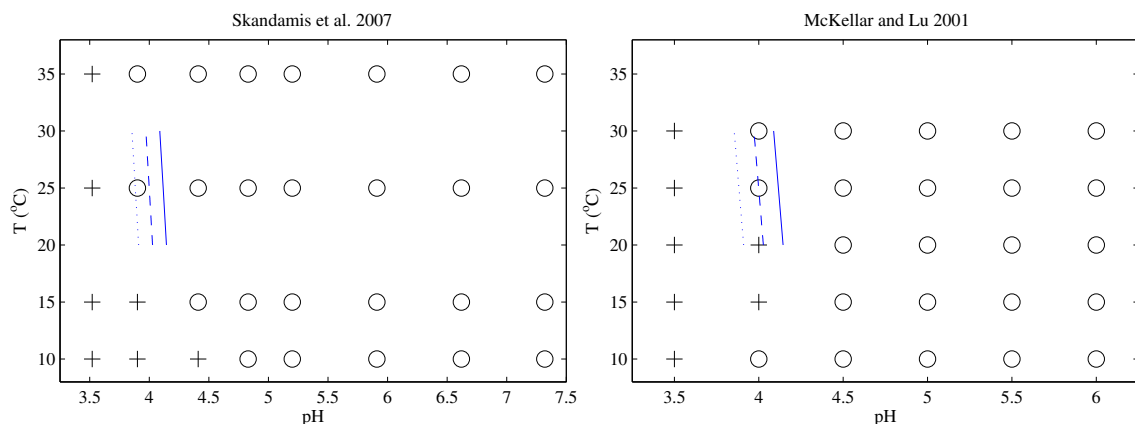


Fig. 3. Predicted growth/no growth interfaces of 10% (···), 50% (---) and 90% (—) for *E. coli* O157:H7 strain ATCC 43888 and observed data from Skandamis et al. (2007) (mix of *E. coli* O157:H7 strains ATCC 43895, ATCC 43889, ATCC 51658 and EO139) and McKellar and Lu (2001) (mix of *E. coli* O157:H7 strains C7927, C9490, 380-94, EC940340, and EC920283).

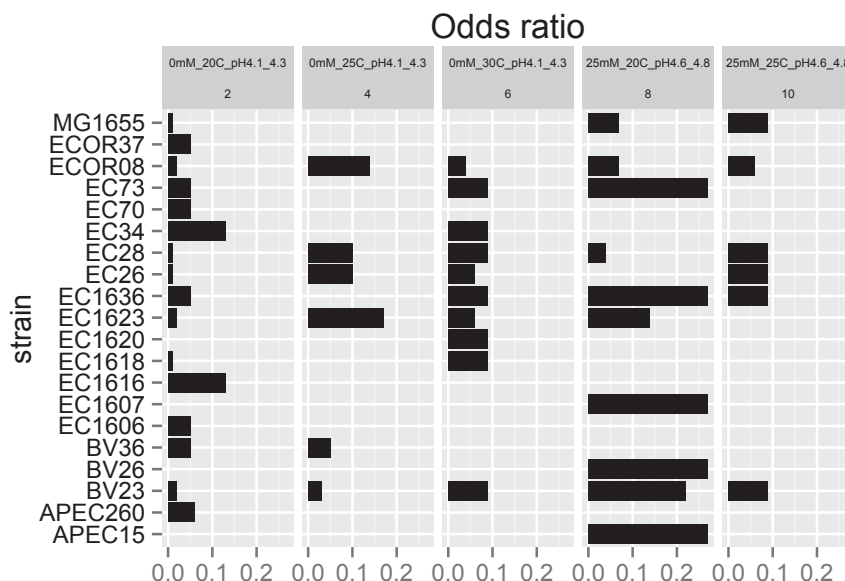


Fig. 5. Odds ratios of observing growth under high pH conditions (conditions 2, 4, 6, 8 and 10) relative to the *E. coli* O157:H7 reference strain for all strains that showed significant growth differences ($p < 0.05$) compared to the reference strain. Condition 12 is not shown because no strains showed significant differences with the reference. Reading key: *E. coli* BV23 (the third strain upward on the figure) has approximately odds of growth 0.075 times lower than *E. coli* O157:H7 in condition 10 (25 mM LA, 25 °C and pH between 4.6 and 4.8) and approximately 0.2 times lower than *E. coli* O157:H7 in condition 8 (25 mM LA, 20 °C and pH between 4.6 and 4.8).

will not be discussed. Considering the environmental factors ranges used in this work, the significant variables selected through the purposeful selection differed and only pH had a significant contribution to the probability of growth for all the strains. The parameter values for pH were always positive (meaning pH has a positive effect in the growth probability) and larger than the others parameters values for all strains. This higher influence on the growth responses was expected because the pH range was very close to the border conditions for 0 and 25 mM LA, differently for temperature which range values were closer to the optimum temperature for this pathogen. *E. coli* optimum temperature for growth (37 °C) was not tested since this value can be lower under acidic pH conditions (Presser et al., 1998), and we wanted to study values closer to ambient temperature considered more relevant in for food context. For all strains (S1), LA parameters were negative and temperature parameters were positive. The narrow increment in pH (0.1 pH units) and the use of single strains permitted a detailed observation of the pH influence on the growth interface.

The variability in the kinetics of growth and survival at low pH among O157:H7 strains is well proven in the literature (Benjamin and Datta, 1995; Large et al., 2005; Lee et al., 2012; Oh et al., 2009; Saridakis et al., 2004). Benjamin and Datta tested the ability of 14 enterohemorrhagic (EHEC) *E. coli* strains (6 being O157:H7) to survive in pH 3.0 and 2.5 at 37 °C, and the strain ATCC 43888 was classified as moderately acid-tolerant (10–50% survival). Oh et al. (2009) observed a significant difference in acid resistance in 400 mM acetic acid solutions among *E. coli* O157:H7 strains isolated from different sources. Non-O157:H7 isolated from bovine feces had a similar behavior to the O157:H7 strains. Large et al. (2005) studied the variability in three acid resistance (AR) systems present in *E. coli* among three groups of Shiga toxin-producing *E. coli* (STEC) clones (O157:H7, O26:H11/O111:H8, and O121:H19) and six commensal strains from ECOR group A. The average survival rate for the O157:H7 group was significantly lower than the STEC strains in two AR systems, and in one of the AR systems, O26/O111 were significantly better than O157:H7 group, leading to the conclusion that this group is not highly acid resistant with these specific AR mechanisms. To our knowledge, data about growth/no growth

variability among O157:H7 strains is not present in the literature. Previous studies mixed different O157:H7 representatives and described the growth/no growth responses, but did not test whether they were different or not (McKellar and Lu, 2001; Skandamis et al., 2007).

Lianou and Koutsoumanis (2011), in a study about the growth variability between 60 isolates of *Salmonella enterica*, observed that strain variability increases as the growth conditions became more stressful in terms of pH and NaCl. The results from the GLMM reinforce the hypothesis that strain variability is more pronounced in stressful pH conditions since the odds ratio from strains significantly different from O157:H7 strain ATCC 43888 was always higher at low pH ranges. The occurrence of strains significantly different from O157:H7 strain ATCC 43888 presenting higher odds of growth indicates that many strains found in the environment can be more resistant to acid conditions than the selected O157:H7 strain. Even if all the strains used in this work were not exclusively clinical or food isolates, it is important to understand the overall strain adaptation response to low pH.

5. Conclusions

The aim of this study was to assess the variability in the growth boundaries of *E. coli* strains according to pH, temperature and lactic acid concentration. The results contribute to the existing knowledge about strains variability by providing clear evidence of variance in the growth boundaries of *E. coli* strains. As far as we are aware our study is the first in the published literature to use such a high number of *E. coli* strains from different sources in growth/no growth experiments (Belessi et al., 2011; Skandamis et al., 2007; Valero et al., 2010; Vermeulen et al., 2007). Depending on the temperature, the pH growth boundaries for the 188 *E. coli* strains ranged between 3.8 and 4.3 with 0 mM LA, and between 4.3 and 4.8 with 25 mM LA. Comparing the odds of growth of *E. coli* O157:H7 strain ATCC 43888 with the other *E. coli* it was possible to observe that the variation in the ability to growth is larger under lower pH conditions than in higher pH conditions tested in this study.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.fm.2014.06.024>.

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